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2-Cyclohexylcarbonylbenzimidazoles as potent, orally available and brain-penetrable opioid receptor-like 1 (ORL1) antagonists

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ABSTRACT

The synthesis and biological evaluation of new potent opioid receptor-like 1 (ORL1) antagonists are presented. Conversion of the thioether linkage of the prototype [It is reported prior to this communication as a consecutive series.: Kobayashi, K.; Kato, T.; Yamamoto, I.; Shimizu, A.; Mizutani, S.; Asai, M.; Kawamoto, H.; Ito, S.; Yoshizumi, T.; Hirayama, M.; Ozaki, S.; Ohta, H.; Okamoto, O. *Bioorg. Med. Chem. Lett.*, in press] to the carbonyl linker effectively reduces susceptibility to P-glycoprotein (P-gp) efflux. This finding led to the identification of 2-cyclohexylcarbonylbenzimizole analogue **7c**, which exhibited potent ORL1 activity, excellent selectivity over other receptors and ion channels, and poor susceptibility to P-gp. Compound **7c** also showed satisfactory pharmacokinetic profiles and brain penetrability in laboratory animals. Furthermore, **7c** showed good in vivo antagonism. Hence, **7c** was selected as a clinical candidate for a brain-penetrable ORL1 antagonist.

A fourth opioid receptor, opioid receptor-like 1 (ORL1), was discovered in 1994 by homology cloning.[2–5] Subsequently, its endogenous agonist, a 17-amino acid peptide termed nociceptin or orphanin FQ (NC/OFQ), was identified.[6,7] Pharmacological studies using NC/OFQ and ORL1-deficient mice showed that the NC/OFQ-ORL1 system may play important roles in the regulation of pain response,[8] morphine tolerance,[9] learning and memory,[10–12] food intake,[13] anxiety,[14] the cardiovascular system,[15,16] locomotor activity,[17] etc.[18] These results prompted many pharmaceutical companies to identify potent and selective antagonists. However, in order to clarify the biological role of the ORL1 receptor and investigate the therapeutic potential of the ORL1 antagonists, there remains a need to develop orally available and brain penetrable ORL1 antagonists.

We previously reported the identification of potent ORL1 antagonists with high selectivity over binding affinity for human ether-a-go-go related gene (hERG).[1] Further evaluation revealed that most compounds with a hydrophilic group on a thioether part related to **1** and **2** exhibited significantly low brain penetrability (Table 1).[19–21] We demonstrated that **1** and **2** were substrates for human and mice P-gp efflux. This suggests that the poor brain penetrability is due to P-gp mediated efflux. We report here structure–activity relationship (SAR) studies to address this issue in the benzimidazole series of ORL1 antagonists.

The syntheses of compounds **7a**–**e** followed the general procedure shown in Scheme 1. Nitroanilines **3** were reduced with Fe followed by condensation with formic acid to give benzimidazole **5**, which was protected using a 2-(trimethylsilyl)ethoxymethyl (SEM) group to afford intermediate **6**. Coupling **6** with the corresponding ester using lithium tetramethylpiperidide (LTMP) followed by deprotection of the SEM group with tetrabutylammonium fluoride (TBAF) provided compounds **7a**–**e**. The starting material **3a** was prepared from 3-fluoro-4-methylaniline **8** via nitration with potassium nitrate in trifluoroacetic anhydride followed by hydrolysis of the resultant trifluoroacetamide.

Cyclohexanone analogue **9** was prepared by a similar coupling reaction as mentioned above, followed by removal of the SEM group and ketal in aqueous TFA (Scheme 2).

As shown in previous communications,[1,22,23] we have already performed structural modification at the piperazine ring, the thioether substituent and 5-substitution on the benzimidazole ring. Therefore, the SAR study was expanded to a hitherto unmodified region, the linkage moiety at the 2-position on the benzimidazole core using compound **1** as a template. Replacing the sulfur atom, however, with methylene, a nitrogen atom and an oxygen atom resulted in complete loss of potency (data not shown). In contrast, ketone analogue **7a** was tolerable in terms of binding affinity for

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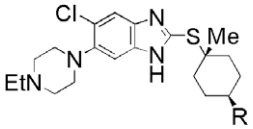
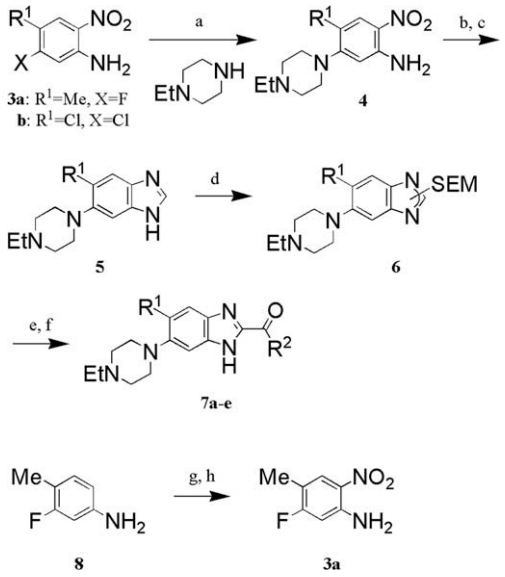
Table 1
Binding affinity for ORL1, brain penetrability, and P-gp susceptibility of **1** and **2**

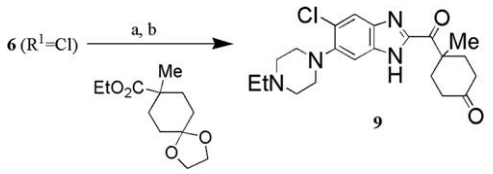


Compds	R	ORL1 binding[a] IC_{50} (nM)	Brain penetrability[b]			P-gp[c] (transport ratio)	
			Plasma (nM)	Brain (nmol/g)	b/p ratio	Human	Mice
1	$NHCO_2Me$	2.4	22.2	1.11	0.05	16	21
2	N(Me)Ac	0.81	4.56	0.38	0.09	33	54

[a] See Ref. 19 for detailed description. $n = 1$ (Ref. 20).
[b] At 1 h after po administration in mice (30 mg/kg).
[c] Transport ratio: B–A/A–B (Ref. 21).



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMSO, 120 °C; (b) Fe, NH_4Cl, THF–MeOH–H_2O, reflux; (c) HCO_2H, 100 °C; (d) SEMCl, NaH, THF, 55–74% (4steps); (e) R–CO_2Et, LTMP, THF, −78 °C, 46–55%; (f) TBAF, THF, reflux, 57–93%; (g) KNO_3, TFAA, rt; (h) K_2CO_3, MeOH–H_2O, rt, 66% (2 steps).

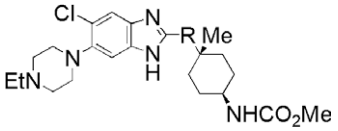


Scheme 2. Reagents and conditions: (a) LTMP, THF, −78 °C, 56%; (b) TFA–H_2O (10:1), rt, 99%.

ORL1 as compared with compound **1** (Table 2).[19–21] Surprisingly, this modification resulted in a marked improvement in susceptibility to human and mouse P-gp-mediated efflux. Although the reason for this difference is not clear, we speculate that it correlates with pK_a value of the benzimidazole.[24] As a new template, 2-carbonylbenzimidazoles resulted from modifying the linkage moiety, and we then revisited the SAR at the benzimidazole and cyclohexane rings.

The results of SAR studies on ORL1 activity, selectivity over hERG binding, human metabolic stability, and the susceptibility to human P-gp efflux are summarized in Table 3.[19–21,25] First, the

Table 2
Effect of the linkage at 2-position on benzimidazole ring



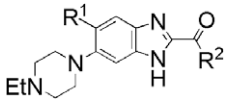
Compds	R^1	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	P-gp[b] (transport ratio)	
				Human	Mice
1	S	2.4	0.72	16	21
7a	C=O	5.7	7.5	1.9	3.6

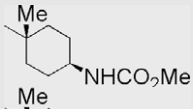
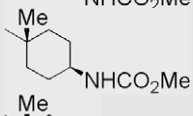
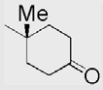
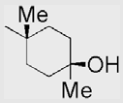
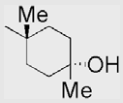
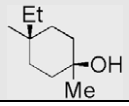
[a] See Ref. 19 for detailed description. $n = 1$ (Ref. 20).
[b] Transport ratio: B–A/A–B (Ref. 21).

effects of substituents at the 5-position of the benzimidazole core were investigated. Replacement of the chlorine atom with a methyl group (**7b**) resulted in a slight increase in potency for ORL1; however, the binding affinity for hERG was also enhanced, despite the decrease in lipophilicity.[26] We next carried out SAR studies to assess the effects of substituents on the cyclohexane ring. Cyclohexanone analogue **9** showed a twofold reduced ORL1 affinity, although affinity for hERG was slightly reduced. Incorporation of the *tert*-alcohol group into the 4-position on the cyclohexane ring led to interesting results with regard to binding affinity for ORL1 and hERG. The trans isomer **7c** exhibited potent ORL1 affinity and markedly improved selectivity over hERG, without impairment of metabolic stability and susceptibility to P-gp. In comparison, compound **7d** with a cis configuration showed higher affinity for the hERG channel, despite the fact that the binding affinity for ORL1 was twenty times lower than that for **7c**. Replacement of the methyl group at the 1-position with a bulky ethyl group (**7e**) resulted in slight enhancement of the binding affinity for ORL1; however, its metabolic stability was decreased due to the increase in the lipophilicity. Consequently, we selected compound **7c** for further evaluation.

As shown in Table 4, compound **7c**[27] was a highly selective ORL1 antagonist against other opioid receptors. In a standard panel for off-target activity, **7c** showed good selectivity over the other 160 receptors and ion channels (adrenergic α1 non-selective (72%), $\alpha 1_D$ (74%), cannabinoid CB_2 (73%), histamine H_3 (50%) and choline transporter (62%) reached over 50% at 10 μM). The potential cardiovascular effects of **7c** were evaluated in anesthetized dogs. At 10 mg/kg iv dosing (C_{max} = 10.7 μM), no adverse treatment-related cardiovascular effects were observed.

Table 3
In vitro profiles of 2-cyclohexylcarbonylbenzimidazoles



Compds	R^1	R^2	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	hERG binding[b] IC_{50} (nM)	log $D_{7.4}$[c]	HM stability[d] % remaining	P-gp[e] (transport ratio)	
								Human	Mice
7a	Cl		5.7	7.5	4900	3.7	74	1.9	3.6
7b	Me		2.0	5.3	3000	3.1	71		
9	Cl		9.9	38	8500	3.0			
7c	Cl		1.4	1.3	20,000	3.1	82	1.7	5.5
7d	Cl		27		3100				
7e	Cl		0.80	2.4	11,000	3.6	57	2.0	3.0

[a] See Ref. 19 for detailed description. $n = 1$ (Ref. 20).
[b] Displacement of a [^{35}S] -radiolabeled MK499 in membranes derived from HEK 293 cells stably transfected with hERG gene and expressing the I_{Kr} channel protein.
[c] Measured by shake-flask method.
[d] See Ref. 25 for detailed description.
[e] Transport ratio: B−A/A−B (Ref. 21).

Pharmacokinetic (PK) studies were carried out in rats, dogs and monkeys, and **7c** exhibited moderate PK profiles. Relatively low clearance and good plasma half-life were observed in dogs and monkeys, despite the high clearance and short half-life in rats.

Table 4
Off-target activities, PK profiles, and brain penetrability of **7c**

Compound		**7c**
Binding IC_{50}	ORL1 (nM)	1.4
	μ[a] (nM)	>10,000
	κ[a] (nM)	1400
	δ[a] (nM)	>10,000
Pharmacokinetics[b]		
Rat	F (%)	15
	$T_{1/2}$ (h)	0.62
	Cl (ml/min/kg)	77
Dog	F (%)	38
	$T_{1/2}$ (h)	3.5
	Cl (ml/min/kg)	34
Monkey	F (%)	8
	$T_{1/2}$ (h)	4.0
	Cl (ml/min/kg)	22
Brain penetrability[c]	Plasma (μM)	1.08
	Brain (nmol/g)	2.21
	b/p ratio	2.02

[a] Displacement of a [^{3}H]diprenorphin (μ), [^{3}H]U69593 (κ), and [^{3}H]naltrindole binding to CHO cells stably expressing cloned human μ-, κ-, and δ-opioid receptors, respectively.
[b] Oral dose 3 mg/kg and IV dose 1 mg/kg.
[c] At 30 min after sc administration in mice (3 mg/kg).

With regard to brain penetrability in mice, the brain–plasma ratio was 2.02, which was dramatically improved as compared with the thioether leads. As **7c** was not subject to human P-gp efflux, it should be a good brain penetrant in humans.

Furthermore, in vivo antagonistic activity of **7c** was tested in the rat carrageenin-induced hyperalgesia model (Fig. 1). This compound, administered at a dose of 30 mg/kg sc, significantly inhib-

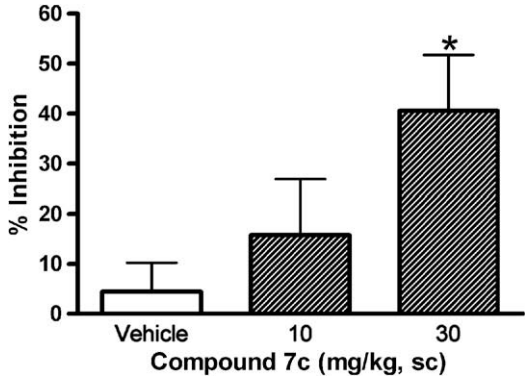


Figure 1. Effect of **7c** on carrageenin-induced hyperalgesia in rats. Carrageenin (0.15 ml, 3%) was administered ipl. 3 h before test. Test was performed 1 h after **7c** injection. Rat's responses to noxious mechanical stimuli were measured. * $P < 0.05$ versus vehicle.

ited the hyperalgesia induced by carrageenin 1 h after administration.

In conclusion, we performed SAR studies, both for ORL1 activity and for susceptibility to P-gp efflux, for a benzimidazole series of ORL1 antagonists related to **1**. Modification of the linkage moiety at the 2-position of the benzimidazole core led to identification of 2-cyclohexylcarbonylbenzimizole **7c**, which exhibited potent ORL1 activity and low susceptibility to P-gp efflux. **7c** shows fair pharmacokinetic properties in rats and dogs, and good brain penetrability in mice. In addition, **7c** was found to be efficacious against the carrageenin-induced hyperalgesia in rats. Based on the profiles described in this report, **7c** was selected as a clinical development candidate for the potential treatment of various CNS dysfunctions. Further developmental progress will be reported elsewhere.

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27. Analytical data of **7c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] 1.00–1.30 (1H, m), 1.16 (3H, t, J = 7.3 Hz), 1.19 (3H, s), 1.30–1.92 (9H, m), 2.45–2.85 (6H, m), 2.54 (2H, q, J = 7.3 Hz), 2.98–3.26 (4H, m), 7.10–7.95 (2H, m), 10.02–10.27 (1H, br). MS (ESI): m/z 419.3 $[M+H]^+$.